

FDA's Role In Building the ID NGS Diagnostic Toolkit

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Principal Investigator**

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Opinions are my own



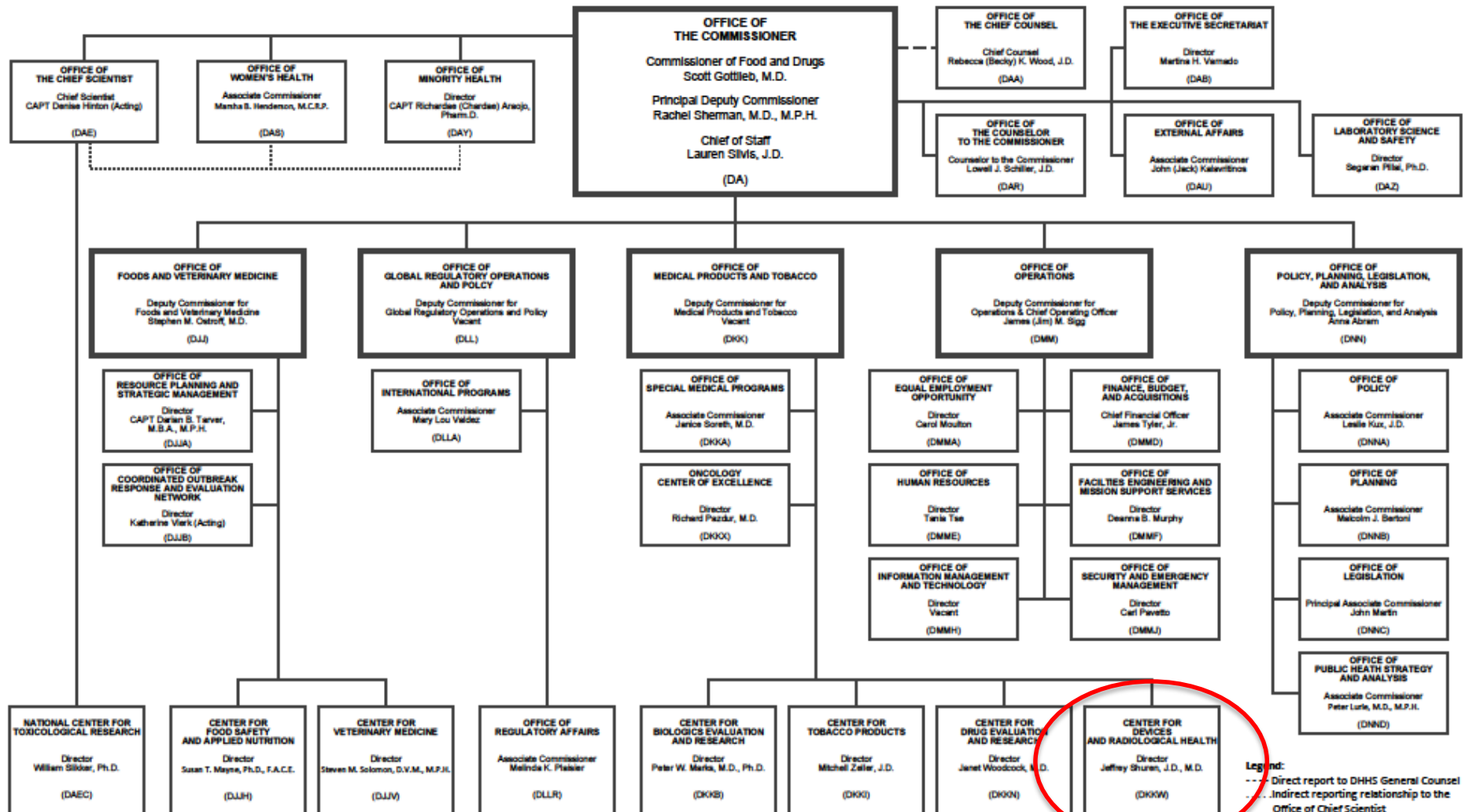


Organizational Chart



FOOD AND DRUG ADMINISTRATION

Approved by the FDA Reorganization Coordinator
& Principal Delegation Control Officer
25 September 2017



FDA White Oak Campus



Resources For You

Medical Devices

- **Emergency Use Authorizations**
 - <https://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm>
- **Device Advice: Comprehensive Regulatory Assistance**
 - <https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/default.htm>
- **Recently-Approved Devices (PMA, 510(k), HDE, De novo)**
 - <https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/Recently-ApprovedDevices/default.htm>
- **Classify Your Medical Device**
 - <https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Overview/ClassifyYourDevice/default.htm>
- **Science and Research (FDA-ARGOS Database)**
 - <https://www.fda.gov/MedicalDevices/ScienceandResearch/DatabaseforReferenceGradeMicrobialSequences/default.htm>

In Vitro Diagnostic Devices



Definition:

Reagents, instruments, and systems intended for use in the diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae. ... for use in the collection, preparation, and examination of specimens from the human body. [21 CFR 809.3]

US FDA Regulated Uses:

- Detection and Diagnosis
- Screening
- First Response
- Not Environmental Screening

Device Classification

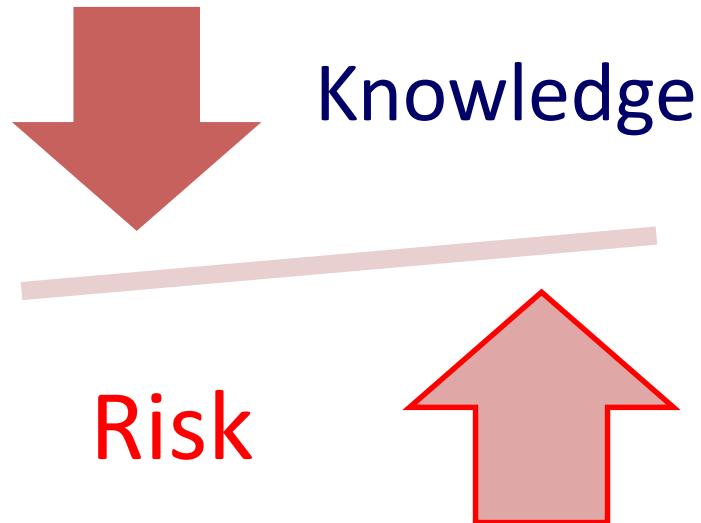


A device should be placed in the lowest class whose level of control will provide reasonable assurance of safety and effectiveness.

Risk Based Regulation of IVDs



Class I most 510(k) exempt



Class III - PMA

Knowledge Mitigates Risk

Class I - Low likelihood of harm
register & list (21CFR §807)

General Controls

Class II - Moderate likelihood of
harm or risk can be
mitigated

Special Controls

Class III - High or unknown
likelihood of harm
Significant Risk

Pre-market Approval

Risk Dependent on Intended Use

Different Use, Same Test

- A CFTR genotyping multiplex assay on the same instrument with the indication
 - ✓ for aid in diagnosis →510(k)
 - ✓ for fetal screening →PMA

- A breast cancer assay to be used
 - ✓ for screening, diagnosis →PMA
 - ✓ for prognosis in already diagnosed patients →510(k)

ID NGS Draft Guidance

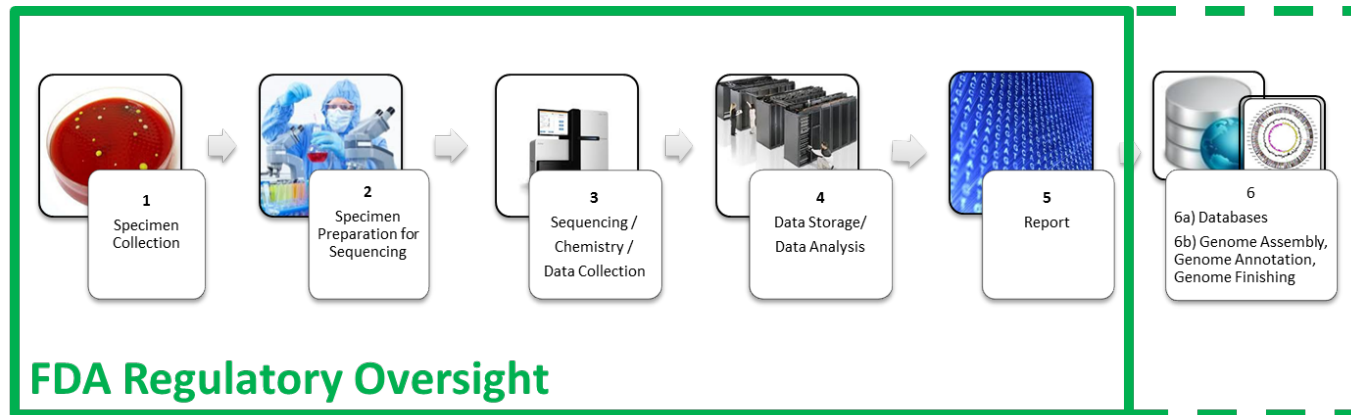
There is **no FDA cleared NGS system for sequencing of microbial genomic DNA** for identification of microbial targets or detection of virulence or resistance genes.



The screenshot shows the Federal Register website interface. At the top, there is a navigation bar with links for Sections, Browse, Search, Policy, Learn, Blog, and My FR. Below this is the Federal Register logo and the text "The Daily Journal of the United States Government". The main heading reads "Infectious Disease Next Generation Sequencing Based Diagnostic Devices: Microbial Identification and Detection of Antimicrobial Resistance and Virulence Markers; Draft Guidance for Industry and Food and Drug Administration Staff; Availability". Below the heading, it states "A Notice by the Food and Drug Administration on 05/13/2016". There is a green button labeled "SUBMIT A FORMAL COMMENT". On the left, under "ACTION", it says "Notice.". Under "SUMMARY", it begins with "The Food and Drug Administration (FDA) is announcing the availability of a draft guidance entitled 'Infectious Disease Next Generation Sequencing Based Diagnostic Devices: Microbial Identification and Detection of Antimicrobial Resistance and Virulence Markers.' This draft guidance provides recommendations to assist industry in designing studies to establish the analytical and...". On the right, there are links for "Previous Document" and "Next Document", a "LEGAL DISCLAIMER" section, "Font Controls" with plus/minus and text color buttons, and a "PUBLIC INSPECTION" section with links for PDF, DEV, and PRINT.



FDA Current Thinking



NGS Technologies

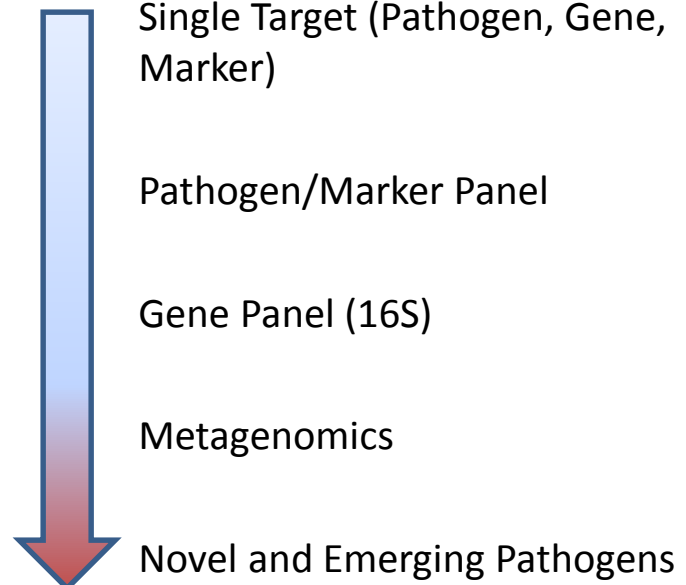
Targeted (*amplicon, bioinformatics*)

- Scope limited to defined regions that target a specific organism(s), gene(s) or marker(s).
- Targets are selected *a priori* by any lab or bioinformatics method (e.g., amplicon sequencing or a k-mer signature database) based on the diagnostic devices intended use.

Hypothesis-Free (*whole genome, shotgun*)

- No *a priori* knowledge of targets.
- Generally can identify all constituents (e.g., organism(s), gene(s) or marker(s), microbiota, human background, and contaminants) in a sample.

Sample Applications



De Novo Regulatory Pathway



The De Novo process provides a pathway to classify novel medical devices for which [general controls](#) alone, or general and [special controls](#), provide reasonable assurance of safety and effectiveness for the intended use, but for which there is no legally marketed [predicate device](#). De Novo classification is a risk-based classification process.

There are two options for when a sponsor can submit a De Novo request for the FDA to make a **risk-based evaluation for classification of the device into class I or II**.

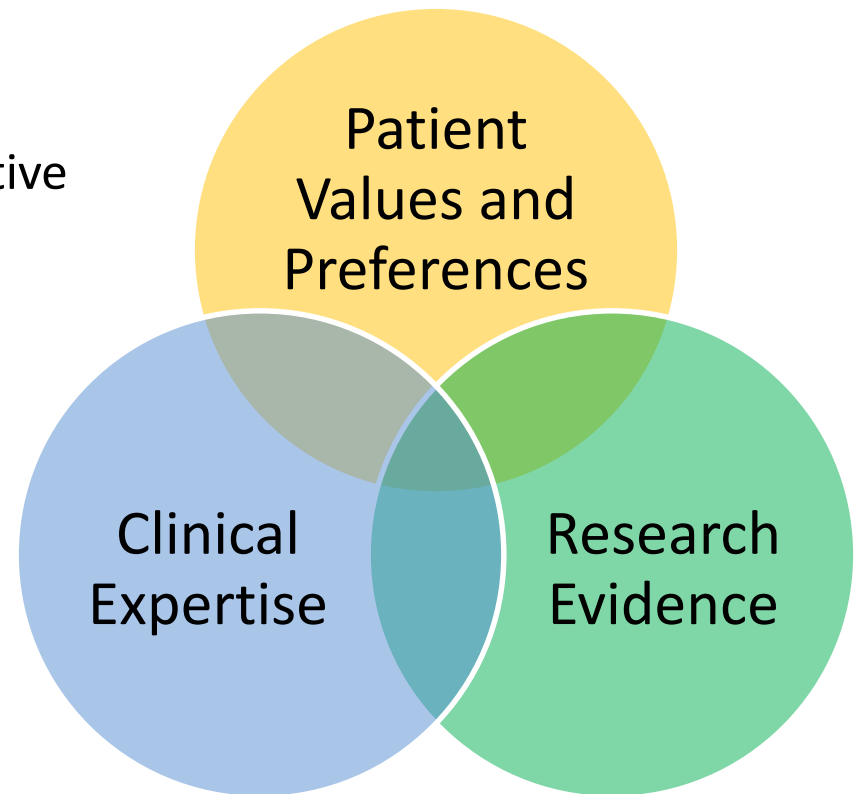
- Option 1: After receiving a high-level not substantially equivalent (NSE) determination (i.e., new intended use and/or different technological characteristics that raise different questions of safety and effectiveness) in response to a 510(k) submission.
- Option 2: Upon the sponsor's determination that there is no legally marketed device upon which to base a determination of substantial equivalence (therefore without first submitting a 510(k) and receiving a high-level NSE determination).

Prior to submitting a De Novo request, it is recommended that you consider submitting a [pre-submission \(pre-sub\)](#) to obtain feedback from the appropriate premarket review division.

[De Novo Classification Process \(Evaluation of Automatic Class III Designation\) - Guidance for Industry and Food and Drug Administration Staff \(PDF - 139KB\)](#)

Risk-Based Evaluation

- Real clinical samples where feasible
 - Prevalence of analyte is low?
Consult with FDA about alternative sample types
- Prospective or retrospective evaluation
 - Comparison to a reference method
 - Comparison to a predicate device
 - Comparison to a clinical outcome
- In-Silico evaluation
 - FDA-ARGOS Reference-Grade Genomes (Bioproject 231221)
 - Mixed Microbial Reference Material



ID NGS Diagnostic Toolkit Needs



- ID NGS Diagnostic Assay
- Tools to support regulatory review
 - **FDA-ARGOS Reference-Grade Genomes** for regulatory use to enable sponsor to perform in-silico validation of claims
 - **Mixed Microbial Reference Materials** that sufficiently challenge the entire ID NGS Diagnostic Assay workflow
 - Ideally cell-based
 - Performance for assay's intended use

FDA Tools for ID NGS Dx



FDA-ARGOS Database

:microbial reference genomes for **regulatory use**

- ✓ **More flexible regulatory pathway**
 - Enable In-silico analytical and clinical validation
 - Reduce testing burden
- ✓ **Reference database**

Interagency ID NGS SME Working Group

: team of NGS agency-wide subject matter experts

- ✓ **ID NGS Dx Advisory Board**
- ✓ **Consensus FDA-ARGOS genome vetting**
- ✓ **Keep current on state of the art**
- ✓ **Tackle open questions (i.e. sens/spec)**
- ✓ **NGS Reference Material**

Reference Genomes For Regulatory Use



Support in-silico analytical and clinical validation

- A. Identified by orthogonal reference method
- B. Sequenced and de-novo assembled using 2 sequencing methodologies
- C. High depth of sequencing coverage
- D. Minimum of 20X over 95 percent of the assembled and polished core genome
- E. Taxonomy-specific ANI thresholds that are sufficient for identification
- F. Placed within a pre-established phylogenetic tree
- G. Sample specific metadata, raw reads, assemblies, annotation and details of the bioinformatics pipeline are available

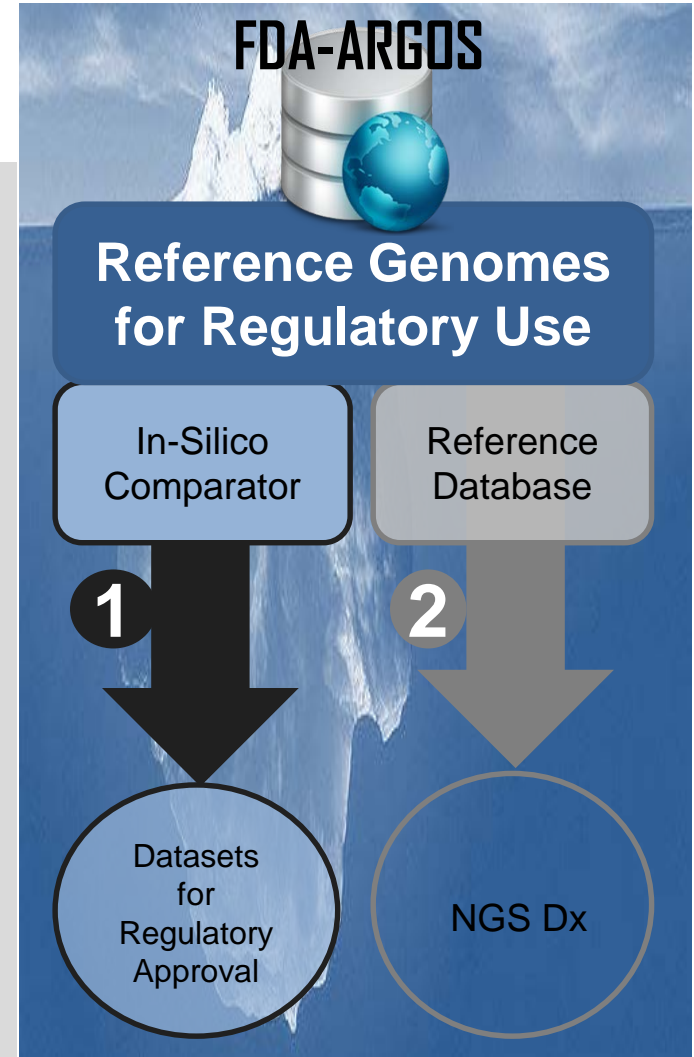
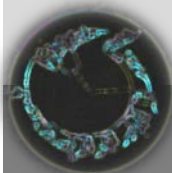


FDA dAtabase for R eference G rade micrObial S equences (FDA-ARGOS)



Government-Academic-Clinical Partnership

- In May 2014, the FDA and collaborators established FDA-ARGOS (www.fda.gov/argos)
- With funding support from FDA's Office of Counterterrorism and Emerging Threats (**OCET**) and **DoD**, the FDA-ARGOS team are initially collecting and sequencing 2000 microbes that include biothreat microorganisms, common clinical pathogens and closely related species.
- Currently, FDA-ARGOS microbial genomes are generated in 3 phases.
 - Phase 1 entails collection of a previously identified microbe and nucleic acid extraction from government, academic and clinical collaborators (>30).
 - Phase 2, the microbial nucleic acids are sequenced and de novo assembled using Illumina and Pac Bio sequencing platforms at the **Institute for Genome Sciences at the University of Maryland (UMD-IGS)**.
 - Phase 3, the assembled genomes are vetted by an ID-NGS subject matter expert working group consisting of FDA personnel and collaborators and the data are deposited in **NCBI** databases.



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In-Silico Comparator Example

DoD/USAMRIID Collaboration

- Sequencing-based diagnostic device
- Generate FDA-ARGOS Reference Genomes
- Datasets for Regulatory Approval

> Enable In-Silico Data Analysis

Endemic African Diseases

Chikungunya virus
Crimean-Congo hemorrhagic fever virus
dengue virus serotype 1
dengue virus serotype 2
dengue virus serotype 3
dengue virus serotype 4
Ebola virus
Lassa virus
Marburg virus (Angola)
Marburg virus (Ci67)
Plasmodium falciparum
Rift Valley fever virus
West Nile virus
Yellow fever virus
Zika virus

FDA-ARGOS Pipeline



FDA-ARGOS microbial genomes are generated in 3 phases:

Phase 1- collection of a previously identified microbe and nucleic acid extraction

Phase 2- sequencing and de novo assembly at UMD

Phase 3- Vetting and data deposit in NCBI databases

FDA-ARGOS Reference Genome Characteristics:

- A. Identified by orthogonal reference method
- B. Sequenced and de-novo assembled using 2 sequencing methodologies
- C. High depth of sequencing coverage
- D. Minimum of 20X over 95 percent of the assembled and polished core genome
- E. Taxonomy-specific ANI thresholds that are sufficient for identification
- F. Placed within a pre-established phylogenetic tree.
- G. Sample specific metadata, raw reads, assemblies, annotation and details of the bioinformatics pipeline are available.

Bacteria/Fungi/Eukaryote Pipeline

Collect samples /grow samples

Extract samples

Q/C extractions at IGS (>10ug cutoff)

Batch and library prep/sequence on Illumina –Megablast QC

Library prep/sequence on PacBio –Megablast QC

Assemble long/short raw reads

Annotate with in-house pipeline for Q/C

Data Analytics Q/C Pipeline at FDA

Register BioSamples and submit raw reads to SRA DB and assemblies to Assembly DB

NCBI annotates genomes

Viral Pipeline

Q/C extracted genomic material at IGS (25ng)

Library Prep/sequence on Illumina

- Shotgun
- Amplicon (may require primer set design –Ebola, Zika)
 - Looking into WNV, Dengue –Broad Institute
- RACE

Assemble raw reads

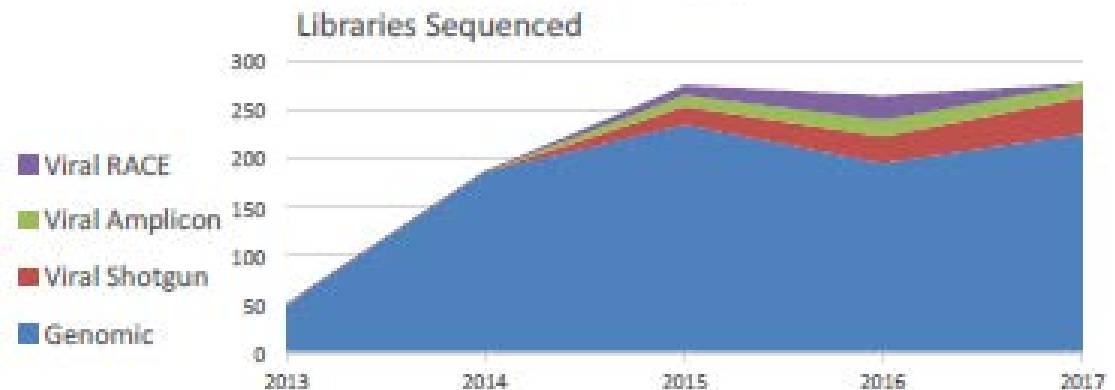
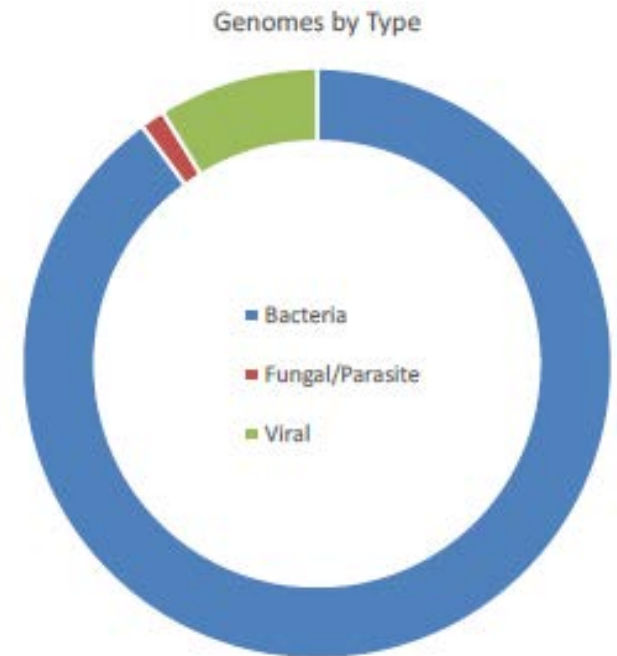
Data Analytics Q/C Pipeline at FDA

Register BioSamples and submit raw reads to SRA DB and assemblies to Assembly DB

FDA-ARGOS Sample Progress



- 970 Genomes sequenced
 - 872 bacterial
 - 95 genera
 - 85 viral
 - 9 viral types
 - Ebola, Zika, Dengue, WNV, etc.
 - 13 fungal/parasite
- 30+ collaborators



Reference Genome Gap: Ebola

Endemic African Diseases

Chikungunya virus
Crimean-Congo
Hemorrhagic Fever virus
Dengue virus serotype 1
Dengue virus serotype 2
Dengue virus serotype 3
Dengue virus serotype 4

Ebola virus

Lassa virus
Marburg virus (Angola)
Marburg virus (Ci67)

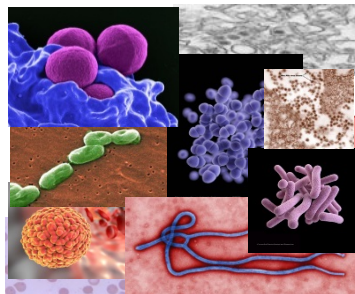
Plasmodium falciparum

Rift Valley fever virus
West Nile virus

Yellow fever virus

Zika virus

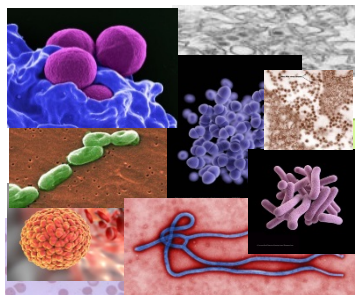
Non-Curated Database



Misdiagnosis:

- ☐ False Positives
- ☐ False Negatives

Standardized Reference Database



Correct Diagnosis:

- ☒ True Positives
- ☒ True Negatives

✓ *Minimize Misdiagnosis*

✓ *Evolutionary Change*

✓ *Rapid Diagnostics*

Sustainable Solution



U.S. Department of Health and Human Services

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Database for Reference Grade Microbial Sequences (FDA-ARGOS)

Facts about FDA-ARGOS

FDA-ARGOS Collaborators

Facts about FDA-ARGOS

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About FDA-ARGOS:

In May 2014, the FDA and collaborators established a publicly available database for Reference Grade microbial Sequences called FDA-ARGOS. With funding support from FDA's Office of Counterterrorism and Emerging Threats (OCET) and DoD, the FDA-ARGOS team are initially collecting and sequencing 2000 microbes that include biothreat microorganisms, common clinical pathogens and closely related species.

Currently, FDA-ARGOS microbial genomes are generated in 3 phases. Generally:

- Phase 1 entails collection of a previously identified microbe and nucleic acid extraction.
- Phase 2, the microbial nucleic acids are sequenced and de novo assembled using Illumina and Pac Bio sequencing platforms at the [Institute for Genome Sciences](#) at the University of Maryland (UMD-IGS).
- Phase 3, the assembled genomes are vetted by an ID-NGS subject matter expert working group consisting of FDA personnel and collaborators and the data are deposited in NCBI databases.

The FDA-ARGOS genomes meet the quality metrics for reference-grade genomes for regulatory use. FDA-ARGOS reference genomes have been de novo assembled with high depth of base coverage and placed within a pre-established phylogenetic tree. Each microbial isolate in the database is covered at a minimum of 20X over 95 percent of the assembled core genome. Furthermore, sample specific metadata, raw reads, assemblies, annotation and details of the bioinformatics pipeline are available.

How FDA-ARGOS Will Assist Medical Device Developers:

Manufacturers can develop sequence-based test to identify infectious agents and/or to detect resistance or virulence markers can use FDA-ARGOS to advance their development programs and to support the regulatory science review of such test. For example, FDA-ARGOS can be used as a tool for in-silico (computer simulation) data analysis.

Contributing Genomes to FDA-ARGOS:

Further population and curation of the database will support the success of FDA-ARGOS and promote adoption by the NGS community. The FDA-ARGOS team openly invites additional collaborators from the scientific community to assist in filling the gaps in this public resource. The FDA-ARGOS and collaborators are specifically searching for unique, hard to source microbes such as biothreat organisms, emerging pathogens, and clinically significant bacterial, viral, fungal, and parasitic genomes. The goal is to collect sequence information for a minimum of 5 isolates per species.

For more information about contributing samples for UMD-IGS sequencing as part of FDA-ARGOS efforts, or to quality existing genomes by the FDA, please email FDA-ARGOS@fda.hhs.gov.

Page Last Updated: 03/22/2017

Note: If you need help accessing information in different file formats, see [Instructions for Downloading Viewers and Players](#).

Language Assistance Available: Español | 繁體中文 | Tiếng Việt | 한국어 | Tagalog | Пускоуст | العربية | Kreyòl Ayisyen | Français | Polski | Português | Italiano | Deutsch | 日本語 | العربية | English

FDA

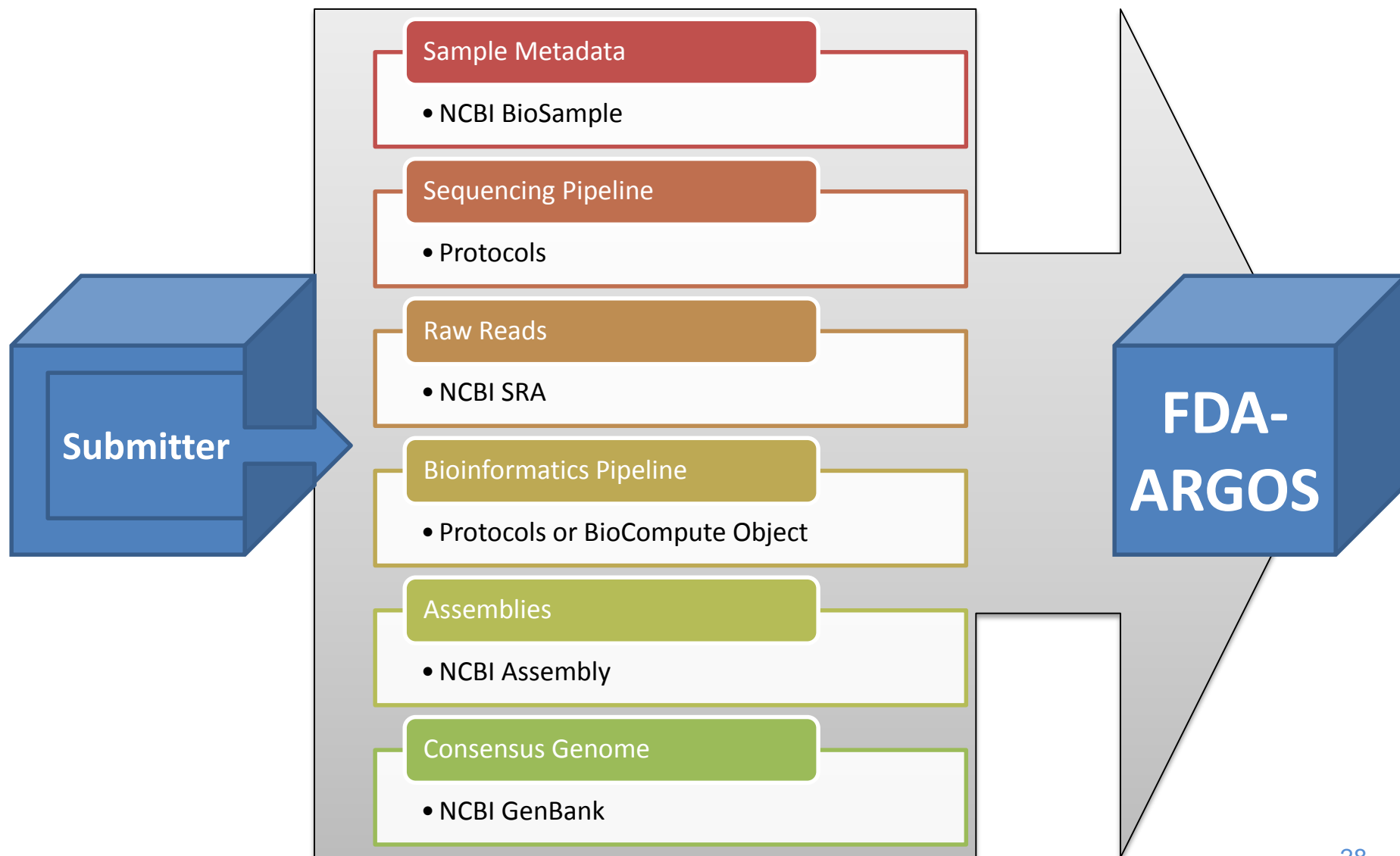
Accessibility | Careers | FDA Basics | FOIA | No FEAR Act | Site Map | Nondiscrimination | Website Policies

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- The goal is to collect sequence information for a minimum of 5 isolates per species.
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External Genome Submission



Reference Materials

- Support analytical validation of entire ID NGS Diagnostic assay workflow
 1. Cell-based in clinical matrix (blood, urine, stool) to test from specimen collection to result
 2. Reference material organism panel should sufficiently capture ID NGS assay's claimed target characteristics (intended use)
 - Size of the genome, G/C content, DNA/RNA, Near neighbors, Repetitive content, Commensal, Extremes
- Cross-platform comparison

NIST/FDA Reference Material Efforts



- Microbial Genomic DNA Reference Material
 - RM 8375 - Microbial genomic DNA standards for sequencing performance assessment
 - 2 FDA-ARGOS strains/ 2 FDA-CFSAN strains
- Mixed Pathogen DNA Research Material
 - A mixture of genomic DNA from 25 clinically-relevant pathogens plus human genomic DNA.

Build Reference Genomes:

- PacBio/Illumina sequencing of microbial constituents as part of FDA-ARGOS project

Other Reference Material Efforts



- ZymoBIOMICS™ Microbial Community Standards by Zymo Research
 - A mock microbial community consisting of eight bacterial and two fungal strains
- UCSF Control Material

Build Reference Genomes:

- PacBio/Illumina sequencing of microbial constituents as part of FDA-ARGOS project



Which of the following are characteristics of Reference-Grade Genomes for Regulatory Use?

- HMW genomic material from unknown organism
- Sequenced and de-novo assembled using 2 sequencing methodologies
- High depth of sequencing coverage
- Minimum of 1X over 95 percent of the assembled and polished core genome
- Generalized ANI threshold
- Placed within a pre-established phylogenetic tree
- Sample specific metadata, raw reads, assemblies, annotation and details of the bioinformatics pipeline are available.





Which of the following are characteristics of Reference-Grade Genomes for Regulatory Use?

- ~~○ HMW genomic material from unknown organism~~
- ✓ Sequenced and de-novo assembled using 2 sequencing methodologies
- ✓ High depth of sequencing coverage
- ~~○ Minimum of 1X over 95 percent of the assembled and polished core genome~~
- ~~○ Generalized ANI threshold~~
- ✓ Placed within a pre-established phylogenetic tree
- ✓ Sample specific metadata, raw reads, assemblies, annotation and details of the bioinformatics pipeline are available.



REVIEW

Characteristics of Reference-Grade Genomes for Regulatory Use

- A. Identified by **orthogonal reference method**
- B. Sequenced and **de-novo** assembled using **2 sequencing methodologies**
- C. **High depth** of sequencing coverage
- D. Minimum of **20X over 95 percent** of the assembled and **polished core genome**
- E. **Taxonomy-specific** ANI thresholds that are sufficient for microbial organism identification
- F. Placed within a **pre-established phylogenetic tree**
- G. **Sample specific metadata, raw reads, assemblies, annotation and details of the bioinformatics pipeline are available**

Acknowledgements

FDA-ARGOS team members include representatives from the:

- U.S. Food and Drug Administration
- U.S. Department of Defense
- National Institutes of Health
- Institute for Genome Sciences at University of Maryland

Funding Agencies

FDA's Office of Counterterrorism and Emerging Threats

Joint Program Executive Office for Chemical and Biological Defense (JPEO-CBD)

American Type Culture Collection/ BEI
Bernard Nocht Institute for Tropical Medicine, Germany
Biodefense and Emerging Infections Research Resources Repository
British Columbia Centre for Disease Control (BCCDC)
Children's National Medical Center
Defense Threat Reduction Agency (DTRA)
George Washington University
Joint Program Executive Office for Chemical and Biological Defense (JPEO-CBD)
Lawrence Livermore National Lab (LLNL)
Los Alamos National Lab (LANL)
Mayo Clinic
National Biodefense Analysis and Countermeasures Center
National Institute of Allergy and Infectious Diseases (NIH-NIAID)
New York State Wadsworth Laboratories
Public Health Agency Canada (PHAC)
Public Health England (PHE)
Rockefeller University
Rutgers University
Stanford University Medical Center
University of California, San Francisco (UCSF)
University of Colorado Denver
University of Ibadan, Nigeria
University of Louisville
University of Michigan
University of North Carolina at Chapel Hill
University of Texas Medical Branch (UTMB)
University of Washington School of Medicine
U.S. Army Edgewood Chemical Biological Center (ECBC)
U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID)
U.S. Food and Drug Administration
Weill Cornell Medicine

